Large homozygous deletions of the 2q13 region are a major cause of juvenile nephronophthisis

Martin Konrad*, Sophie Saunier*, Laurence Heidet, Flora Silbermann, France Benessy, Joaquim Calado, Denis Le Paslier1, Michel Broyer2, Marie-Claire Gubler and Corinne Antignac*

INSERM U 423, Hôpital Necker-Enfant Malades, 75743 Paris Cedex 15, France, 1CEPH, 27 rue Juliette Dodu, 75010 Paris, France and 2Service de Néphrologie, Hôpital Necker-Enfants Malades, Université René Descartes, 75743 Paris Cedex 15, France

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Juvenile nephronophthisis (NPH) is a genetically heterogeneous disorder representing the most frequent inherited cause of chronic renal failure in children. We recently assigned a gene (NPH1) to the 2q13 region which is responsible for approximately 85% of cases. Cloning this region in a yeast artificial chromosome contig revealed the presence of low copy repeats. Large-scale rearrangements were detected in 80% of the patients belonging to inbred or multiplex NPH1 families and in 65% of the sporadic cases. Surprisingly, these rearrangements seem to be, in most cases, large homozygous deletions of approximately 250 kb involving an 100 kb inverted duplication. This suggests a common genetic disease-causing mechanism, which could be responsible for the highest frequency of large rearrangements reported in an autosomal recessive trait. Our findings are also of major clinical interest, as they permit the diagnosis in the majority of sporadic cases without the need for kidney biopsy.

INTRODUCTION

Familial juvenile nephronophthisis (NPH) or recessive medullary cystic kidney disease is an autosomal recessive kidney disorder leading to end-stage renal disease during childhood or adolescence. The underlying pathology is a chronic tubulo-interstitial nephropathy with characteristic tubular basement membrane thickening and medullary cyst formation (1). NPH represents the most frequent inherited cause of chronic renal failure in children (2). Associations with various extrarenal symptoms, especially ocular lesions, are frequently observed (reviewed in ref. 3). By means of linkage analysis, it has been shown that a gene (NPH1), responsible for the vast majority (approximately 85%) of the purely renal form of NPH, maps to chromosome 2q13 (4–6). By haplotype analysis we have identified two DNA markers flanking the NPH1 gene at loci D2S1890 and D2S1888 (7). This interval of approximately 2 cM and the surrounding region was cloned in a yeast artificial chromosome (YAC) contig. The region turned out to be partially duplicated on chromosome 2p12. Furthermore, several markers mapped to more than one locus within the NPH1 region on 2q13 suggesting the presence of low copy repeats (Fig. 1A).

As low copy repeats have been shown to predispose to large-scale chromosomal rearrangements (reviewed in ref. 8), we performed PCR analysis of sequence tagged sites (STSs) mapping to the region, on patients’ DNA to detect possible abnormalities such as aberrant or absent PCR products. Here, we report the detection of absent PCR amplification products in the majority of NPH patients, indicating rearrangements. Subsequent analysis by Southern blotting and pulsed field gel electrophoresis (PFGE) demonstrated large homozygous deletions. The telomeric deletion breakpoint maps to the distal element of an approximately 100 kb inverted duplication.

RESULTS

PCR analysis

No abnormal patterns were observed in any of the patients for the polymorphic markers which were tested initially (D2S1890, D2S340, D2S1889, D2S1891, D2S1893 and D2S1888) (7). In a second attempt, we tested probands from 13 inbred families for 10 non-polymorphic markers mapping to the critical NPH1 interval between D2S1890 and D2S1888 (Fig. 1A). These markers were obtained mainly by YAC end sequencing during the contig construction (7) or by screening DNA sequence databases for D2S1735 and WI5972. No amplification product was detected using primers generated from the left insert end of YAC 765F2L (Table 1) in affected individuals from 11 of the 13 consanguineous families. We subsequently tested individuals from 13 non-consanguineous NPH1 multiplex-families and 23 sporadic cases. In 10 of 13 and 15 of 23 of these sets, respectively, no PCR amplification product was obtained with the 765F2L primers in affected children (Fig. 2). This is consistent with the expected frequency of rearrangements deduced from the results obtained...
Detailed physical map of the NPH1 deletion region. (A) Minimal YAC contig spanning the disease locus. YAC clones are presented as solid lines. Vertical traits indicate positive STSs. The YAC ends mapping to the region are shown as diamonds. The open square along the schematic chromosome indicate the critical NPH1 interval taken from the genetic map (7). The low copy repeats are typed in bold. The order of the STSs had been determined with additional YAC clones (7). Brackets covering the cluster D2S340–D2S1889–877F8R indicate that the exact order is unknown. Cen, centromere; tel, telomere. (B) Higher resolution and physical organization of the region surrounding the deletions detected in the patients. The positions of additional markers and the restriction sites (ClaI, C; FspI, F; SalI, S; SacII, Sc) are indicated to scale. The inverted duplication is shown as bars. (C) Approximate location of the common 250 kb deletion. Symbols are: open boxes, size of the deletion; hatched boxes, breakpoint region; filled boxes, non-deleted region.

in consanguineous families. The lower proportion of rearrangements in sporadic cases is in agreement with the 10–15% of NPH patients unlinked to NPH1. In contrast, an amplification product was detected in all parents (n = 61), all healthy sibs (n = 41), in a large cohort of control subjects (n = 203), and in the NPH patients belonging to the four families unlinked to NPH1. To study the extent of these rearrangements, we tested flanking markers already mapped to the region (876H12L, 921H4R, W15972, 851H5R) (7) as well as additional markers generated by vector-arm PCR (804H10R) or inter-Alu PCR (804/6) (Table 1) by PCR on patients’ DNA. In all patients lacking the 765F2L amplification product, the 804/6 band was also absent. In only one patient did the rearrangement also encompass the marker 804H10R. All other markers revealed normal amplification products.

Detection of deletions by Southern blotting

Using the purified 765F2L amplification product from normal individuals as a probe for Southern blot analysis, 7.0 and 4.8 kb EcoRI fragments were detected in the genomic DNA in all healthy controls (n = 50) and unaffected family members (n = 45) tested. In contrast, the 4.8 kb band was found to be absent in all affected individuals from 15 families who had no amplification product for 765F2L (Fig. 3). As hybridization to a cell hybrid panel revealed that the 7.0 kb fragment is located on chromosome 1 and the 4.8 kb on chromosome 2 (data not shown), a simple polymorphism could be ruled out. Thus, the absent band demonstrates the presence of a homozygous deletion.
Table 1. Novel STSs mapping to the NPH1 region, developed by YAC end sequencing or inter-Alu PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer sequence</th>
<th>Amplified product size (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>765F2L</td>
<td>GCT CCT TCC TGA GAA GAC AG</td>
<td>142</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>CCA CCT CTC ATC CAG ACA CT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>804H10R</td>
<td>TGA AGG TAG TGT TGT AGA GG</td>
<td>142</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>GCC CAT TTT GAC AGT TTT G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>804/6</td>
<td>TTA AGC CCA AGT AAC CAT AGT C</td>
<td>215</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>GAC AAA TGG AAT TAA CGA AAT A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Southern blot analysis of EcoRI-digested DNA probed with 765F2L. The genomic probe, derived from YAC end sequencing, detected two restriction fragments of 7.0 and 4.8 kb. The absence of the chromosome 2 specific 4.8 kb band in the affected individuals indicates a homozygous deletion.

Long-range restriction mapping

To elucidate the physical organization of NPH1 candidate region, we performed long-range restriction mapping of the YACs covering the region using PFGE and field inversion gel electrophoresis (FIGE). We used the restriction enzymes SaII, SacII, SfiI and ClaI for single and double digestions. The markers 921H4R and WI5972 which were thought to lie telomeric to 765F2L after STS content mapping, turned out to flank the deleted region on both sides indicating a duplication. The repeats were distinguished by the detection of two restriction fragments of different sizes for markers WI5972, 921H4R and 769G7R in YACs covering the whole region (876H12, 879D3 and 804H10), whereas only one fragment was detected in YACs distal to the 804/6 marker (765F2, 769G7 and 921H4). The positions of the restriction sites and markers provided evidence for a large inverted duplication of approximately 100 kb (Fig. 1B).

Size of the deletion

To determine the size of the deletions, genomic DNA from 18 unrelated patients (13 of whom carry the homozygous deletion) and six controls was digested with NotI and separated by PFGE. A 790 kb fragment was detected, containing 804/6 and 765F2L (which are deleted in the patients) as well as the flanking markers 804H10R and 921H4R, in normal controls (Fig. 4). This enabled us to characterize the size of the deletion. A fragment length difference of approximately 250 kb was observed between the 13 patients already known to be deleted by PCR or Southern blot analysis versus controls and patients who were not thought to be deleted (Fig. 4). In addition, three patients were found to be heterozygous for the deletion. Even though a larger deletion of one of the alleles could not be completely ruled out, this possibility seems unlikely, as the PCR analysis did not detect a larger deletion in the homozygous state for the flanking markers in any of the patients. Hybridization of patients’ DNA after SfiI digestion with the 921H4R probe revealed the absence of the normal 65 kb fragment in all the 11 deleted patients tested. In contrast, the normal 95 kb fragment was detected in all patients (data not shown). This indicates that the telomeric copy of the duplication is at least partially missing.

DISCUSSION

This study demonstrates large homozygous deletions to be responsible for the majority of cases of juvenile NPH without extrarenal manifestations. The sizes of the deletions seem to be very similar in most patients (approximately 250 kb). Additionally, the deletions involve, at least partially, the telomeric copy of an inverted duplication. Assuming that the breakpoints of these deletions are identical (they have not yet been cloned), this unusual high deletion rate might be due to a founder effect. A common haplotype did not emerge significantly from haplotype analysis of the flanking markers in the NPH outbred population.
(in 26 NPH1 families; data not shown). However, the possibility of a common ancestor cannot be definitely ruled out, as the two closest polymorphic markers flanking the deletion are, at least, 1 Mb apart. It has been shown that, even in a homogeneous population, no significant linkage disequilibrium could be demonstrated for loci located, respectively, 900 and 1600 kb away from the disease gene (9). It is more likely that these rearrangements occurred independently, due to a common mechanism. There are examples of human chromosomal rearrangements in which low copy repeats or duplicated regions predispose to deletion and/or duplication events (8). However, these reports almost always concern autosomal dominant or X-linked disorders. Large rearrangements as a common disease-causing mechanism, have been reported for instance on chromosome 17p where a duplication results in Charcot–Marie–Tooth disease type 1A (10); whereas patients with a deletion of the same region have hereditary neuropathy with liability to pressure palsies (11). Large molecular deletions have also been identified in more than 85% of patients with X-linked ichthyosis (12). Large-scale rearrangements have rarely been shown to be a common cause of autosomal recessive diseases. In familial growth hormone deficiency type 1A homozygous deletions have been described (13) and ‘hot spots’ for recombination leading to growth hormone deficiency type 1A homozygous deletions have been identified (14). However, these deletions have been identified in consanguineous families. In spinal muscular atrophy (SMA), large deletions have been described in a small study were in consanguineous families. In spinal muscular atrophy (SMA), large deletions have been described in a small proportion (seven of 201) of families (15), but a marked heterozygosity deficiency indicates deletions in at least 18% of patients with Werdnig-Hoffmann disease (SMA type I). These findings contributed considerably to the identification of two genes, implicated in SMA (17,18). These genes were subsequently found to be at least partially deleted in a homozygous state in most of the patients studied.

Within the context of repeated sequences triggering rearrangements, it has to be noted, that the telomere–telomere fusion point of the two ancestral ape chromosomes, from which the human chromosome 2 arose (19), has been located to the band 2q13 (20). This fusion point is organized as an inverted array of TTAGGG repeats, which are typical of human telomeres (21). As these repeats, even in recessive traits. The further characterization of large scale rearrangements in regions containing low copy repeats, even in recessive traits. The further characterization of these rearrangements will help in the understanding of the mechanism for these deletions.

This study underlines the usefulness of a systematic search for large scale rearrangements in regions containing low copy repeats, even in recessive traits. The further characterization of these rearrangements will contribute to the isolation of the disease-causing gene or genes in NPH.

The detection of these deletions is also of great clinical interest, as they will contribute to the diagnosis of the most frequent cause of chronic renal failure in children, without invasive diagnostic procedures such as kidney biopsy in sporadic cases, and enable the unequivocal identification of children at risk in NPH families.

MATERIALS AND METHODS

PCR and Southern blot

The polymorphic microsatellite markers mentioned in Figure 1A were amplified using conditions as described in ref. 7. Southern blots were used according to standard laboratory protocols.

YAC-end probe generation

YAC end sequences were amplified by anchored PCR using the primers described by Kere et al. (24). PCR products were directly sequenced after purification or cloned using the TA cloning kit (Invitrogen, San Diego, CA, USA) and subsequently sequenced on an automated sequencer (Applied Biosystems, Foster City, CA, USA) after plasmid DNA isolation (RPM-Kit, Vista, CA, USA). Primer sequences and annealing temperatures for the STSs shown in Figure 1B are taken from ref. 7 or are listed in Table 1 for new markers.

Inter-Alu PCR

Inter-Alu PCR was performed with the primers as described (25) following DNA preparation and amplification conditions proposed by Lengauer et al. (26). PCR products were sequenced after cloning in the TA cloning kit.

Long range restriction mapping

Agarose embedded high molecular weight yeast or human DNA was digested with SauI, SacII, SfiI, ClaI and NotI. Double digestion of DNA from YAC clones with SacII/ClaI, SacII/SalI, ClaI/SfiI, SalI/SfiI were also performed. Fragments were separated through 1% agarose gels in 0.5×TBE buffer using a CHEF DRII electrophoresis system (Biorad, Richmond, CA, USA) for large fragments at the following conditions: Fragments >1 Mb: 200 V with a 60–120 s switch time for 24 h; fragments with a size of 0.1–1 Mb: 200 V with 10–40 s switch time (24 h). For details see ref. 27. Small fragments (5–100 kb) were separated by field inversion gel electrophoresis (FIGE Mapper, Biorad, Richmond, CA, USA) under the following conditions: forward 180 V, reverse 120 V with 0.1–2 s switch time for 16 h at 20°C. Filters were hybridized as described (27) either with the purified STS amplification products (804H10R, 804/6, 765F2L, 921H4R and W15972), or with the cloned YAC end product, when primers were not available (769G7R).
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